PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 12185280/TDO/LM	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. PCT/AU2003/000388	International Filing Date *(day/month/year)* 28 March 2003	Priority Date *(day/month/year)* 28 March 2002

International Patent Classification (IPC) or national classification and IPC

Int. Cl. [7] A61K 38/43, A61K 38/18, A61K 38/19, A61K 38/00, A61P 29/00, A61P 35/00, A61 37/00

Applicant

MEDVET SCIENCE PTY.LTD. et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheet(s).

3. This report contains indications relating to the following items:

   I [X] Basis of the report

   II [ ] Priority

   III [ ] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

   IV [ ] Lack of unity of invention

   V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI [X] Certain documents cited

   VII [ ] Certain defects in the international application

   VIII [ ] Certain observations on the international application

Date of submission of the demand	Date of completion of the report
17 October 2003	9 July 2004
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer M. Ong Telephone No. (02) 6283 2491

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU2003/000388

I. Basis of the report

1. With regard to the **elements** of the international application:*

- [ ] the international application as originally filed.
- [X] the description, pages **1-58,** as originally filed,
  pages , filed with the demand,
  pages , received on with the letter of
- [X] the claims, pages **59-64** , as originally filed,
  pages , as amended (together with any statement) under Article 19,
  pages , filed with the demand,
  pages , received on with the letter of
- [X] the drawings, pages **1/21-19/21,** as originally filed,
  pages , filed with the demand,
  pages **20/21, 21/21,** received on **7 July 2003** with the letter of **7 July 2003**
- [ ] the sequence listing part of the description:
  pages , as originally filed
  pages , filed with the demand
  pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

- [ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- [ ] the language of publication of the international application (under Rule 48.3(b)).
- [ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- [ ] contained in the international application in written form.
- [ ] filed together with the international application in computer readable form.
- [ ] furnished subsequently to this Authority in written form.
- [ ] furnished subsequently to this Authority in computer readable form.
- [ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- [ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:
   - [ ] the description, pages
   - [ ] the claims, Nos.
   - [ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU2003/000388

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 4, 5, 9, 10,11, 15, 16, 19, 26, 27, 31, 32, 35, 42, 43, 48, 49	YES
	Claims 1-3, 6-8, 12-14, 17, 18, 20-25, 28-30, 33, 34, 36-41, 44, 45-47	NO
Inventive step (IS)	Claims 48, 49	YES
	Claims 1-47	NO
Industrial applicability (IA)	Claims 1-49	YES
	Claims	NO

2. Citations and explanations (Rule 70.7)

The following documents identified in the International Search Report have been considered for the purposes of this report:

D1: WO 1999/12533 A
D2: WO 2001/85953 A
D3: Blaukat, A et al.
D4: Machwate, M et al.
D5: Cuvillier, O et al.
D6: Johnson, KR et al.
D7: Maceyka, M et al.

New Citation
D8: Xia, P et al. Sphingosine kinase interacts with TRAF2 and dissects tumour necrosis factor-α signalling. Journal of Biological Chemistry, 8 March 2002, vol. 277(10), pages 7996-8003

Novelty (N): Claims 1-47

D1 discloses a method and agents for modulating cellular activity. Methods of treatment or prophylaxis of a disease condition involving inflammatory mechanisms using an agent capable of modulating one or more components of a sphingosine kinase signalling pathway wherein the modulation results in modulation of adhesion molecule expression, is taught. In particular, HDL treatment of endothelial cells is disclosed to substantially blunt the amplitude and duration of Sph-1-P formation by inhibiting sphingosine kinase activity. This results in the blunting of MEK/ERK activation and NF-κB nuclear translocation thereby reducing adhesion protein expression. N,N-dimethyl sphingosine decreases TNF-α induced adhesion protein expression and mRNA levels by competitively inhibiting sphingosine kinase activity. This is relevant to claims 1-3, 6, 7, 9, 12-14, 17, 18, 20, 21-25, 28-30, 33, 34, 36-41, 44 and 45.

D2 teaches a method of modulating the growth of a cell by contacting the cell with an effective amount of an agent under conditions to modulate the functional activity of sphingosine kinase (SPK). A method of down-regulation of cell proliferation wherein the cell is a neoplastic cell, is disclosed. Antagonists of sphingosine kinase include N,N-dimethyl sphingosine and DL-threo-dihydrosphingosine. Chemical agonists include chemical and functional equivalents of sphingokinase nucleic acid or protein molecules or derivatives produced by common molecular techniques. This is relevant to claims 1-3, 9, 12-14, 20-24, 28-30, 36-38, 44 and 45.

D3 discloses the activation of sphingosine kinase by bradykinin B_2 receptor via activation of ERK/MAP kinase. DL-threo-dihydrosphingosine, a known sphingosine kinase inhibitor was taught to block S1P generation and reduced the B_2 receptor induced ERK and ERK/MAP kinase activation in a dose dependent manner. This is relevant to claims 1-3, 6-9, 12-14, 17-20, 28-30, 33-36, 44 and 45.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of V

D4 teaches the stimulation of cytosolic sphingosine kinase activity by forskolin whilst PD98059, a selective inhibitor stimulates apoptosis in two osteoblastic cell lines. N,N-dimethyl sphingosine, another inhibitor of SPK was shown to completely reverse the antiapoptotic effect of forskolin. Other activators of SPK taught include PDGF, serum and 12-O-tetradecanoylphorbol-13-acetate (TPA) and cAMP. This is relevant to claims 1-3, 6-9, 11-14, 17-22 and 27.

D5 discloses the positive regulation of SPK by 12-O-tetradecanoylphorbol-13-acetate, and is negatively regulated by dimethyl sphingosine. It is further taught that S-1P generated through a protein kinase C mediated activation of SPK, can inhibit apoptosis. This relevant to claims 1-3 and 12-14.

D8 teaches TNF or overexpression of TRAF2 was capable of activating SPK and that TNF-induced SPK activation was blocked by the dominant-negative TRAF2. SPK mutants lacking either the TRAF2-binding motif or enzyme catalytic activity abrogated the effect of TRAF2. This is relevant to claims 1-3, 46 and 47.

Therefore it is considered that claims 1-3, 6-8, 12-14, 17, 18, 20-25, 28-30, 33, 34, 36-41, 44 and 45-47 do not meet the requirements of Article 33(2) PCT with regard to the requirement for novelty in view of the disclosures of D1-D5 and D8.

Claims 4, 5, 9-11, 15, 16, 19, 26, 27, 31, 32, 35 42, 43, 48 and 49 meet the criteria set forth in PCT Article 33(2) for novelty. The prior art published before the priority date does not disclose the modulation of sphingosine kinase functional activity where the modulation of phosphorylation of the sphingosine kinase activity occurs at S^{225}. The prior art, further do not disclose the modulation of said phosphorylation as modulation of proline-directed protein kinase catalysed phosphorylation ie. ERK2. Further, use of U0126 and PD98059 for the treatment and/or prophylaxis of a condition characterised by aberrant , unwanted or otherwise inappropriate sphingosine kinase functional activity where modulation of phosphorylation of sphingosine kinase is warranted were not disclosed.

Inventive Step (IS): Claims 1-47
As above.

Industrial Applicability: Claims 1-47
Claims 1-47 have industrial applicability

Please see indication contained in Box VI, "Certain documents cited" with regard to D6 and D7.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU2003/000388

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

Application No. Patent No.	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date (valid claim) *(day/month/year)*
WO 2002/098458	12 December 2002	3 June 2002	7 June 2001

WO 2002/098458 discloses a method of modulating cytokine-induced cellular activity to modulate interaction of SPK with a TRAF whereby inducing SPK and TRAF association with an agent that binds, links or otherwise associates with the C-terminal region of sphingosine kinase, up-regulates cellular activity and, antagonising said association down-regulates cellular activity. Treatment and/or prophylaxis of conditions characterised by aberrant, unwanted or otherwise inappropriate cytokine-mediated cellular activity with said agent is further taught.

Please refer to the supplemental Box for further comments on D6 and D7.

With regard to the document listed in Box VI, this document was published after the priority date of the present application but would otherwise be considered of particular relevance.

2. Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure *(day/month/year)*	Date of written disclosure referring to non-written disclosure *(day/month/year)*

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

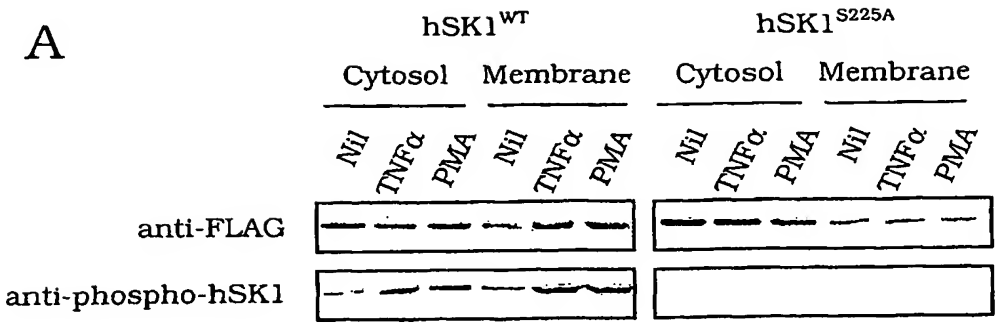
Continuation of VI

D6 teaches the regulation of SPK with the protein kinase C (PKC) activator, phorbol 12-myristate 13-acetate (PMA) through the phosphorylation of SPK.

D7 discloses the known SPK inhibitors threo-dihydrosphingosine (DHS) and NN-dimethylsphingosine (DMS) as well as a list of agonist, amongst others, G-protein coupled receptors (GPCR), including acetylcholine, prosaposin and others. Agonists of growth factor receptor tyrosine kinase are also taught to activate SPK. It is further disclosed that S1P activates ERK in Swiss 3T3 fibroblasts and TNF-α activates ERK in a SPK -dependent manner in U937 leukemia cells. Inhibition of ERK activity by PD98059 is disclosed.

Please note that this opinion has been based on the assumption that the claimed subject matter of the present application validly derives its priority claim. However, D6 and D7 would be relevant to claims 1-3, 6-14, 17-25, 27-30, 33, 41 and 43-45 if the present application is found to not validly claim its priority.

Under the PCT, novelty is considered only in respect of documents published before the priority date. The relevance of a document published after the priority date is dependent upon national law. Such documents are excluded from consideration in preliminary examination, under the PCT Guidelines but have been included here for information


A
hSK1WT
hSK1S225A
Cytosol
Membrane
Cytosol
Membrane
Nil
TNFα
PMA
Nil
TNFα
PMA
Nil
TNFα
PMA
Nil
TNFα
PMA
anti-FLAG
anti-phospho-hSK1

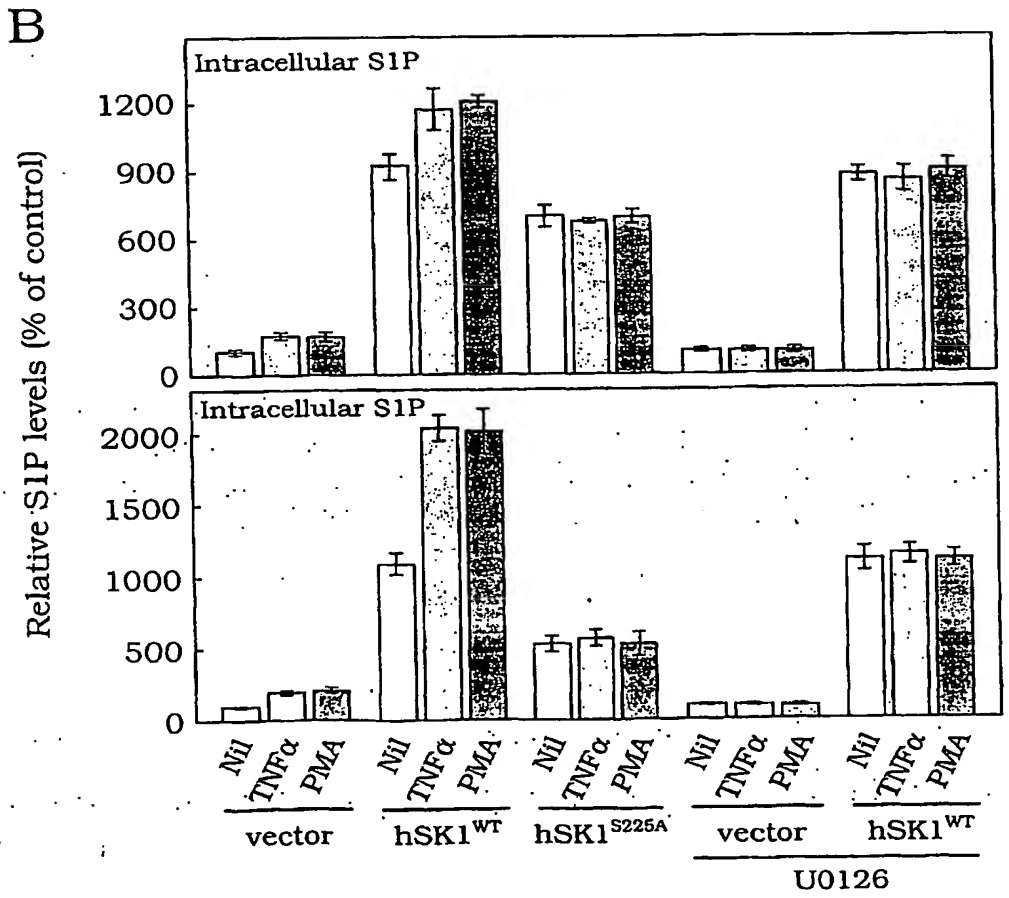
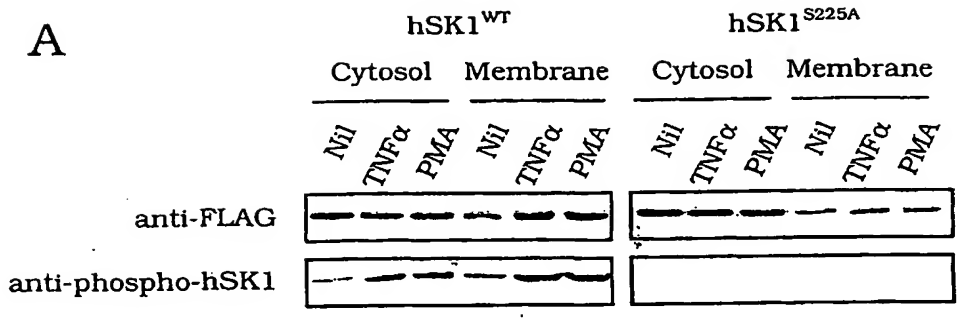

B
Intracellular S1P
Intracellular S1P
Relative S1P levels (% of control)
1200
900
600
300
0
2000
1500
1000
500
0
Nil
TNFα
PMA
vector
hSK1WT
hSK1S225A
vector
hSK1WT
U0126

Figure 18


A
hSK1WT
hSK1S225A
Cytosol
Membrane
Cytosol
Membrane
Nil
TNFα
PMA
anti-FLAG
anti-phospho-hSK1

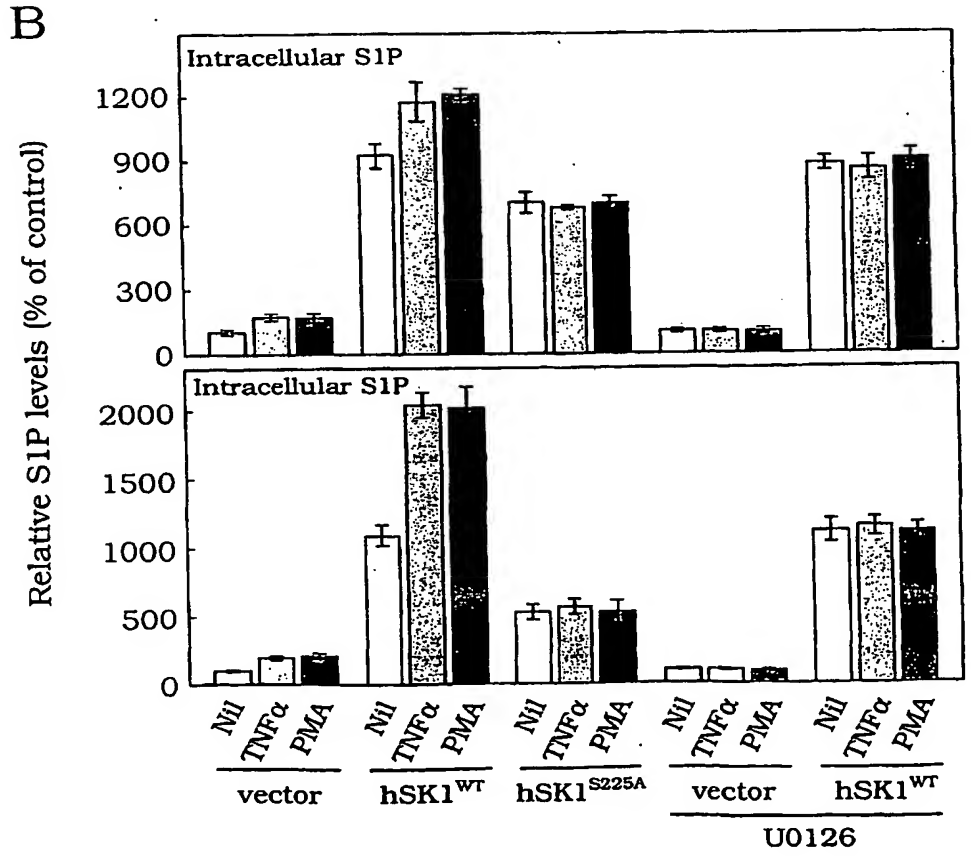

B
Intracellular S1P
Relative S1P levels (% of control)
1200
900
600
300
0
2000
1500
1000
500
vector
hSK1WT
hSK1S225A
vector
hSK1WT
U0126

Figure 18

Received 7 July 2003

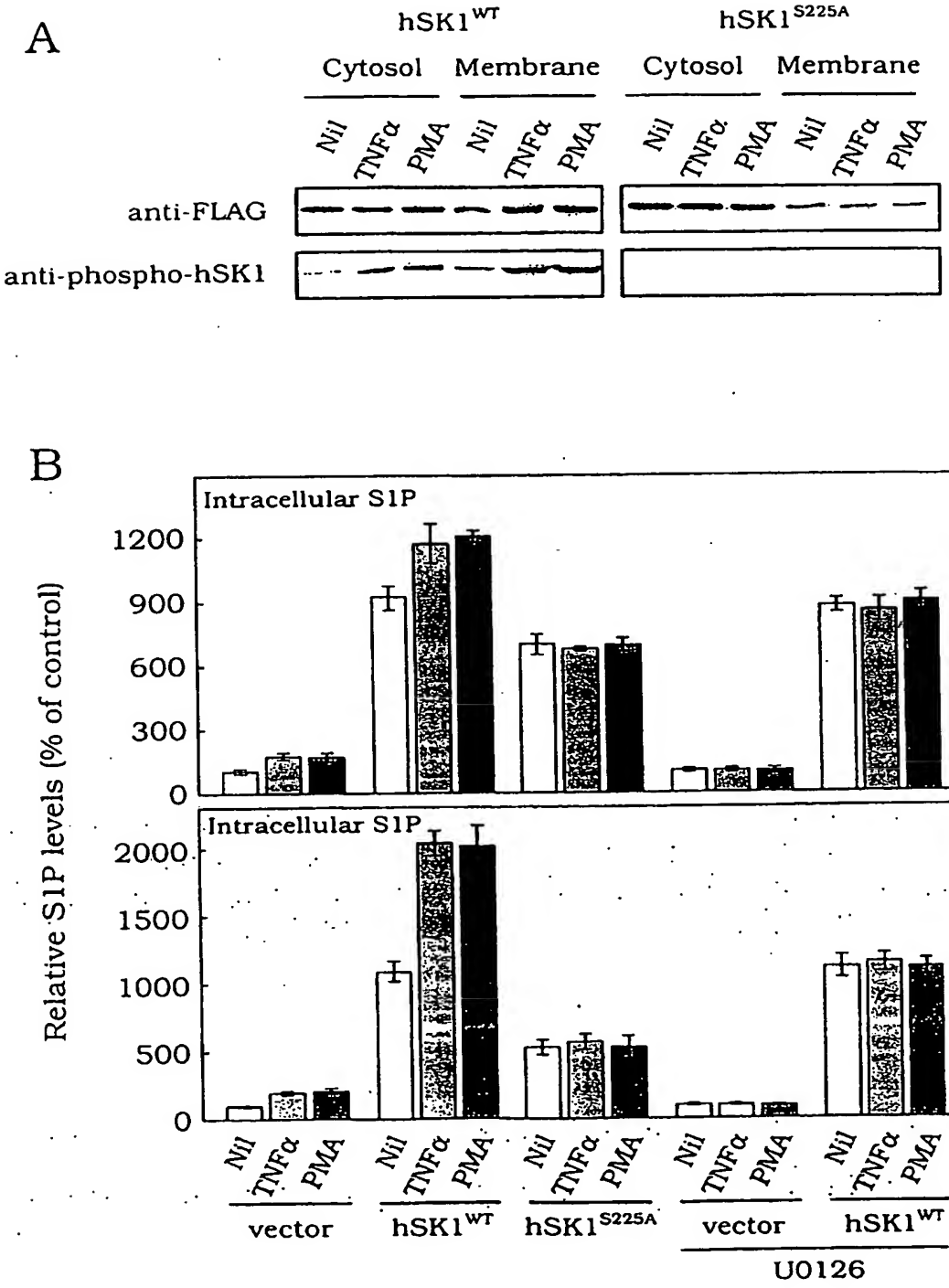


Figure 18

Received 7 July 2003

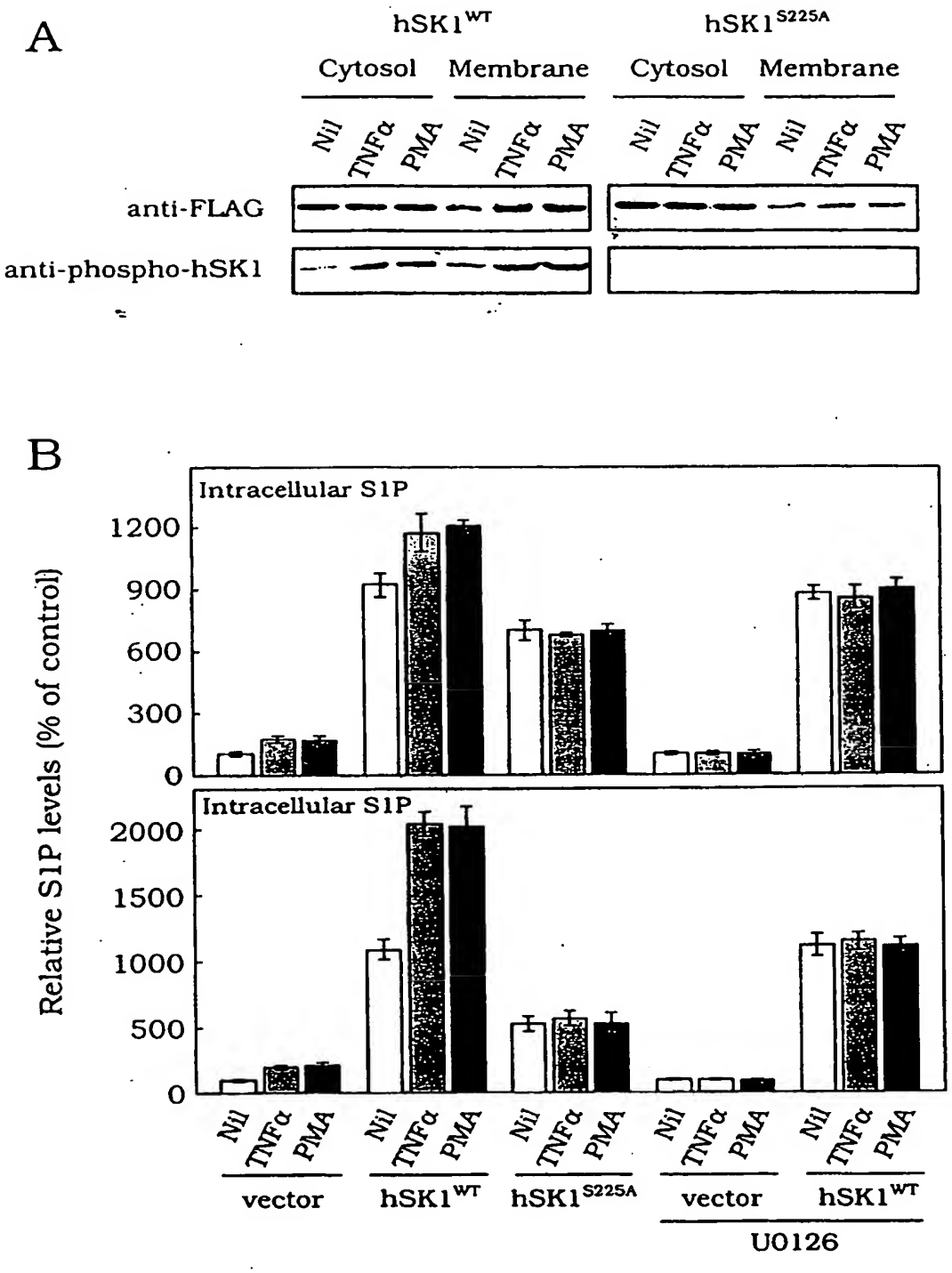


Figure 18

Filing Office Munich

File Number : 03745226.5

EPO - Munich
58
29. Okt. 2004

Date of Receipt : .....

SEQL disc (EP)

EPO - Munich
58
29. Okt. 2004

Sent on : .....

To : Peter Keasberry
(Den Haag - Room S02N16)

FRANK B. DEHN & CO.

Patent and Trade Mark Attorneys

179 Queen Victoria Street, London EC4V 4EL, England
Telephone: +44 (0)20 7206 0600 Fax: +44 (0)20 7206 0700
E-mail: mail@frankbdehn.com Web: www.frankbdehn.com

CONFIRMATION OF FAX

European Patent Office
D-80298 München
Germany

date 28 October 2004
your ref
our ref 27.68.85733

BY FACSIMILE

Dear Sirs

European Patent Application No. 03745226.5 (PCT/AU03/00388)
Medvet Science Pty. Ltd.

I enclose herewith a Form 1200 in respect of the entry into the regional phase before the EPO, together with a fee voucher requesting withdrawal of the necessary fees from our deposit account No. 28050069. If the amount shown is incorrect then please credit or debit the deposit account accordingly.

The proceedings before the EPO are to be based on the form on which the International Preliminary Examination Report is based. A copy of the annexes to the International Preliminary Examination Report is enclosed herewith. Only Figure 8 (on pages 20/21 and 21/21) was amended during International Examination.

I also enclose a copy of the sequence listing on disk for this application and confirm that the sequence information is identical to the written sequence listing.

Please acknowledge safe receipt of this letter and enclosures by date-stamping and returning the attached Form 1037.

Yours faithfully,
Frank B. Dehn & Co.

Hanna Dieglewska

Enc/cst